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# THE ACTION OF BASES AND SALTS ON BIOCOLLOIDS AND CELL-MASSSES.

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(Read April 21, 1921.)

The suggestion was made in an earlier paper that the common metals which enter into nutrient solutions might find their chief importance in restricting, limiting or defining hydration of the cell-colloids.<sup>1</sup> MacDougal and Spoehr carried out a series of tests upon this matter and found in fact that the strong metallic bases when used at concentrations of 0.01 *N* do limit or restrict the hydration of agar according to their place in the electromotive series, the least swelling taking place under the action of the strongest base, with rubidium unplaced. Beginning with the strongest the series runs K(Rb)NaLi, and if calcium were added to the series the swelling under its action was less than that in potassium.<sup>2</sup>

When the concentrations were reduced however to 0.001 *N* it was found that hydroxides of all of the metals increased the hydration capacity of agar. This was of importance as a review of all of the available data seems to show that the range of the  $H^+OH^-$  balance in the plant cell lies between the values expressed by  $P_{H3}$  and  $P_{H11}$ , or between about 0.01 *M* aspartic, succinic or propionic acid and under 0.001 *N* KOH.

The reversal of effects at great attenuations in the hydroxides led to the extension of auxographic measurements upon the effects of low concentrations of the salts which are of such interest and importance in cultures, and the action of chlorides, nitrates and sulfates of potassium, calcium, sodium and magnesium upon agar, gelatine and mixtures was made at Carmel in the summer of 1920.

<sup>1</sup> MacDougal, D. T., "Growth in Organisms," *Science*, 49: 599-605, 1919. (See page 11 of reprint.)

<sup>2</sup> MacDougal, D. T., and H. A. Spoehr, "The Components and Colloidal Behavior of Plant Protoplasm," *Proc. Amer. Phil. Soc.*, 59: 154, No. 1, 1920.

A preliminary announcement of the fact that excessive hydration values in agar and biocolloids were obtained when these substances were swelled in dilute salt solutions was made before the Physiological Section of the Botanical Society of America at Chicago, December 28, 1920, which is in press in the *American Journal of Botany* for June, 1921. Also of interest in this connection is the announcement of Loeb of the reversals of specific effects of salts above and below  $M/16$  on the swelling, osmotic pressure, and viscosity of gelatine.<sup>3</sup>

S. C. J. Jochems measured the effects of a number of acids, bases and salts upon stems of *Laminaria*, *Fucus*, agar, carrageen and upon a number of seeds in 1919. The wealth of results include a number of important generalizations as to the behavior of the plant mucilages and organs when swelled in a number of solutions with a wide range of concentrations.

Jochems found that no rule could be formulated for the influence of the basicity of the acid radicals in the swelling of agar and that the effect of the valency of the base was very small. Still more surprising is the conclusion reached by Jochems that while nearly all of the salts tested, NaCl, NaBr, NaI, NaNO<sub>3</sub>, Na<sub>2</sub>SO<sub>4</sub>, Na<sub>2</sub>HPO<sub>4</sub>, CaCl<sub>2</sub>, lessened swelling at 0.01 *M*, at 0.05 *M* the imbibition was greater than in water. The agar was commercial material and some of the differences between these conclusions and the facts discussed in the present paper are to be attributed in part to my use of a specially purified agar.<sup>4</sup>

My own experiments were planned to test the action of salts of interest in connection with nutritive solutions within the range of biological interest, which would lie between 0.01 *M* and 0.0001 *M*. Single series of swellings in KOH and HCl were included for purposes of comparison.

The first series of tests were made with two plates of agar a year old. Plate *A* swelled 1,800 per cent. in thickness and 3 to 4 per cent. in length when freshly made, and Plate *B*, 3,000 per cent. in

<sup>3</sup> Loeb, J., "The Action of Salts in Low Concentration," *Jour. Gen. Physiol.*, 3: 391-414, 1921.

<sup>4</sup> Jochems, S. C. J., "De imbibite van plantaardige celwanden in oplossen van electrolyten." Published by A. H. Kruyt, Amsterdam, 1919. See pages 35-46.

thickness and 3 per cent. in length. Increases in thickness and volume of sections of the two plates at 14–15° C. are given below. The agar used was of a specially purified lot which had been freed from salts and diffusible carbohydrates by dialysis, and a thin solution was sprayed into 10 times its volume of neutral acetone. The resulting fine shreds were subsequently extracted with hot absolute acetone, absolute alcohol and absolute ether.

TABLE I.

	0.01 M.				0.001 M.				0.0001 M.			
	A		B		A		B		A		B	
	Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.	Th.	Vol.
Water .....	1,460	1,610	1,740	1,990								
KOH .....	715	635	550	605	1,570	1,700						
HCl .....	—	—	760	880	—	—	1,630	1,800	—	—	2,260	2,540
KCl .....	1,140	1,305	1,420	1,565	1,570	1,730	2,500	2,800	1,720	2,000	2,400	2,645
NaCl .....	1,000	1,145	1,160	1,325	1,355	1,520	2,300	2,680	1,750	2,040	2,700	2,975
MgCl <sub>2</sub> .....	750	824	750	824	1,100	1,340	1,470	1,590	1,630	1,925	2,100	2,315
CaCl <sub>2</sub> .....	640	705	725	830	900	945	1,100	1,190	1,500	1,751	1,950	2,160

The accelerating effect of the potassium hydroxide in its weakest, and its retarding effect at its strongest concentrations, confirms previous results by Spoehr and MacDougal. This maximum effect is in a 0.001 *M* solution with a  $P_H$  value of 11. A lesser swelling takes place in either a weaker or a stronger solution.

If we now pass to the acid reactions it will be seen that in HCl at 0.001 *N* with a  $P_H$  value of 3 the swelling of the agar is little short of that in water. At some point between this concentration and 0.0001 *N* swelling becomes equivalent to that which might take place in water and at the last named concentration with a  $P_H$  value of 4.2 the hydration is much in excess of that in water, being as 128 with that in water taken as 100.

The purified agar used has a  $P_H$  value of 6.5 when made up at 0.75 per cent., which is near the limit of its gelation at 15° C. in the

<sup>5</sup> MacDougal, D. T., and H. A. Spoehr, "Hydration Effects of Amino-compounds," *Proc. Soc. Exp. Biol. and Med.*, 17: 33–36, 1919. "The Components and Colloidal Behavior of Plant Protoplasm," *Proc. Amer. Phil. Soc.*, 59, No. 1, 150, 1920. "Swelling of Agar in Solutions of Amino Acids and Related Compounds," *Bot. Gaz.*, 70: 268–278, 1920.

making of the plates which furnished the sections used in the swelling tests. It is seen therefore that the swelling of agar shows a maximum in a hydrogen ion concentration greater than its own as well as in the hydroxide solution at  $P_H$  11.

The above results are to be applied in correction of statements made in many previous publications as to the retarding action of the hydrogen ion on the swelling of pentosans. This mistaken conclusion has been most recently made in the Report of the Department of Botanical Research of the Carnegie Institution of Washington for 1920, pp. 54 and 55, in which it is said that "The pentosans are weak acids and in general their hydration capacity is lessened by hydrogen ions. Hydroxyl ions and compounds containing the amino-groups, such as may be in solutions of phenylalanin, alanin, asparagin and glycocoll, may exert an effect by which hydration capacity is increased above that in pure water. Mucilages derived from various sources show some differences in reactions to the solutions named while conforming to the generalizations given. Their hydration is but little affected by the presence of the common sugars in the water of suspension or dispersion."

Some differences in the reactions of the different plant mucilages to the action of hydrogen and hydroxyl ions might reasonably be attributed to the varying acidity of these substances. Thus commercial acacia gum (gum arabic) and purified cherry gum in a 1 per cent. solution have a  $P_H$  value of 5.1, which is not far from that of a sample of "Bacto" gelatine in an 8 per cent. solution. The mucilage of *Opuntia*, which is taken to form an actual part of the plasmatic mass in the cells of this cactus has a  $P_H$  value of 5.8 as determined by the indicator method.

The data presented in the foregoing table show that the chlorides of the four metals at 0.0001  $M$  with a  $P_H$  value ranging from 5.6 to 6, cause an excessive hydration, which in terms of water as 100, would be  $CaCl_2$ , 110;  $MgCl_2$ , 116;  $KCl$ , 113;  $NaCl$ , 150. At 0.001  $M$   $KCl$  which has a  $P_H$  value of 5.8 as compared with a value of 5.7 at 0.0001  $M$  also causes an excessive hydration. Marked differences are shown by the sections from the two plates in solutions in this concentration, the swelling of one in the sodium being excessive and that of the other lessened. It is to be noted that in the

hydroxides at 0.01 *N* the metals exert a lessening effect on hydration in a series which runs Ca, K, Na with the least swelling in the calcium. In the chlorides at 0.01 *M* the series runs Ca, K, Na, a coincidence strongly suggestive of the specific action of the bases or cations, which has eluded many experimenters.

Sections of the agar Plate *B* were also swelled in nitrates and sulfates of sodium and potassium at 14–15° C. with increases as noted in Table II.

TABLE II.

	Sodium.		Potassium.	
	0.01 <i>M</i> .	0.0001 <i>M</i> .	0.01 <i>M</i> .	0.0001 <i>M</i> .
Sulphates .....	695–740	2,110–2,215	670–710	2,280–2,515
Nitrates .....	905–990	2,470–2,720	780–827	2,640–2,910
Water .....	2,170–2,400	and	2,440–2,560	

It is to be seen that swelling in the sulfates does not exceed the amount possible in water even in the dilute solution, while at 0.0001 *M* the swelling in the nitrates of both sodium and potassium is in excess of that possible in water.

The effect of the salts on gelatine is one which has received attention at the hands of many investigators, but the recently published results of Loeb on the action of these substances at the low concentrations which may be of biological interest are the most decisive yet available.<sup>6</sup> However, it was deemed important to carry out swellings of sections of this substance by the auxographic method in order to secure data strictly comparable with those obtained from the tests with agar. The gelatine was of a sample which, made up in an 8 per cent. solution, had a *P<sub>H</sub>* value of 5.2 Sections 0.27 mm. in thickness were swelled at 14–15° C. and increases were noted in Table III.

The hydration of gelatine as illustrated by the action of the HCl is increased by H or OH ions, the effect rising with the departure from the isoelectric point. Thus the swelling in the acid at 0.0001 *M* *Ph* value of 4.2 is scarcely more than in water, while at 0.01 *M* with a *Ph* value of 2.01 the swelling is over four times as great as

<sup>6</sup> Loeb, J., "The Action of Salts in Low Concentrations," *Jour. Gen. Physiol.*, 3: 391, 1921.

in water alone. The swelling in KCl at 0.0001 *M* with a PH value of 5.7 is not much greater than in water, and the accelerating effect does not rise so rapidly as in the acid solution, the swelling at 0.001 *M* with a PH value of 5.8 being not much more than double that in water. Still another effect of interest is that of the calcium chloride solutions, which induce a maximum swelling at 0.001 *M* but depress hydration as the concentration rises, and as it falls away from this point.

TABLE III.

	0.01 <i>M</i> .		0.001 <i>M</i> .		0.0001 <i>M</i> .	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
KCl.....	1,080	2,100	800	1,400	640	1,160
CaCl.....	600	670	1,350	1,596	830	1,000
HCl.....	1,620	4,680	1,600	2,200	925	1,080
Salt solution (see p. 11).....					730	1,300
Water.....					780	910

The chief interest in all of the foregoing results lies in their possible use in interpretation of the action of living matter. Varied and extensive series of tests have proved that mixtures of pentosans or mucilages and of albumin or gelatine formed a biocolloid in which many of the reactions of living and dead cell-masses to hydration agencies might be exemplified. It was therefore believed to be of importance that the action of salts upon these mixtures should be tested in connection with a measurement of their action upon living material. As has been discussed in many previous papers the behavior of a biocolloid to a hydrating solution depends in many important features upon the proportions of the two main constituents. The action of salts was therefore tested upon two types of biocolloids, one in which the pentosan agar formed the greater proportion and another in which gelatine was the dominant component. The swelling increases of two such mixtures are given in Table IV.

It is notable that in the agar-gelatine mixture the effect of the potassium chloride is essentially identical with that produced on agar alone, except that the limiting effect at the higher concentration is less marked, being at 0.01 *M* but little short of the swelling in water. Sensitiveness to hydrogen ion concentration as shown in reactions to the acid was much more marked than in the agar

alone, and the swelling even in the greatest attenuation of the acid was much less than in water. The limiting effect of the calcium chloride was also very marked.

TABLE IV.

HYDRATION OF MIXTURES OF AGAR 3 PARTS, GELATINE 2 PARTS AT 14° C.

Plates 0.18 mm. in thickness; swelling of sections given in thickness and in volume.

	0.01 <i>M.</i>		0.001 <i>M.</i>		0.0001 <i>M.</i>	
	Th.	Vol.	Th.	Vol.	Vol.	Th.
HCl .....	550	600	930	1,025	1,430	1,575
KCl .....	1,900	2,015	2,270	2,530	2,440	2,640
CaCl <sub>2</sub> .....	920	960	1,220	1,345	2,030	2,268
Water .....					2,200	2,330

TABLE V.

HYDRATION OF MIXTURES OF GELATINE 3 PARTS, AGAR 2 PARTS AT 14° C.

Plates 0.18 to 0.19 mm. in thickness; swellings given in thickness and in volume.

	0.01 <i>M.</i>		0.001 <i>M.</i>		0.0001 <i>M.</i>	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
HCl .....	1,200	1,320	650	690	860	920
KCl .....	800	880	900	1,010	1,620	1,850
CaCl <sub>2</sub> .....	710	740	870	940	1,300	1,430
Water .....					1,275	1,420

The gelatine-agar mixture being a dominantly albuminous mixture, swelling in acid increased with the concentration which was carried to a PH value of 2.01. On the other hand potassium chloride exerted an effect parallel to that shown by its action on agar, the greatest swelling taking place at the lowest concentration with a PH value of 5.7, the increase being much greater than in the acid at the higher concentration.

In general the living cell masses taken from growing organs are dominantly pentosan, but some material has been examined in which the hydration reaction is that of a dominantly albuminous biocolloid. No conception of living matter in plants not including some of the all-pervading common salts is possible, and any attempt to make a complete picture of the colloidal material of the cell must



take into account the compounds of the fatty acids with the common bases, the soaps which as McBain and Salmon have recently shown may exist as both electrolytes and colloids in colloidal masses.<sup>7</sup>

These soaps are an almost inevitable component of protoplasm, and some studies of their possible action in the cell will be taken up in a paper now in preparation. Preliminary to any profitable consideration of the soaps it is necessary to have some definition of the parts which salts may play in the biocolloidal machine. The measurements of swellings given in earlier papers showed that the incorporation of nutritive salts in colloidal masses lessened the hydration capacity. It is now apparent from the results given on the following pages that such restrictive action was due to the high concentrations employed. This however cannot be said of the amino-compounds, which used as hydrating solutions accelerated swellings, but which incorporated in colloidal masses uniformly reduced hydration capacity in whatever concentration used.

In the tests which are to be described it was planned to include the salts which are of importance in nutrition, which induce accelerated swelling in agar and agar-gelatine mixtures in implied concentrations, the calcium and sodium furthermore being used in approximately balancing proportions. These salts were first used with purified agar and hydration values as in Table VI were obtained.

TABLE VI.

## HYDRATION OF AGAR AND SALTS IN SALT SOLUTIONS.

5 g. agar, 100 c.c. KCl 0.001*M*, 60 c.c. NaCl at 0.0001*M*, and 10 c.c. CaCl at 0.0001*M*. Sections 0.2 mm. to 0.27 mm. in thickness swelled at 14° C.

	Th.	Vol.
KCl 0.0001 <i>M</i> .....	2,220	2,780
NaCl 0.0001 <i>M</i> .....	1,800	
MgCl <sub>2</sub> 0.0001 <i>M</i> .....	1,380	
CaCl <sub>2</sub> 0.0001 <i>M</i> .....	1,400	
HCl 0.0001 <i>N</i> .....	2,230	
Salt solution as above .....	2,600	
Water .....	1,760	

The total swelling in the present instance is one which is equivalent to that shown by many preparations including that of agar

<sup>7</sup> *Jour. Amer. Chem. Soc.*, 42: 426, 1920.

Plate *A* with which the salt tests described in the previous pages were made. With reference to the hydrogen ion concentration the solution of salts added to the agar had a PH value of about 5.8 to 6, the agar alone being about 6.5.

The salted agar showed a swelling in even the attenuated solutions of calcium and magnesium chloride less than in water. Such solutions accelerate swelling in pure agar. The swelling of the salted agar in sodium solution was as 102 with water at 100, while that in the potassium solution was 132. That this inequality is not simply a matter of hydrogen ion concentration is evinced by the fact that the swelling in the acid at PH 4.2 was practically equivalent to that of the potassium chloride at 5.7.

The incorporated solution may be regarded as approximately balanced and when the sections were swelled in a similar solution the maximum of the series was reached, the increase being as 150 to water as 100.

An identical solution was used in making up "Bacto-gelatine" and the results of the hydration of sections of the dried plates are as in Table VII.

Hydration of gelatine plate made up gelatine 5 g., 10 c.c. KCl at 0.01 *M*, 6 c.c. NaCl at 0.001 *M*, and CaCl<sub>2</sub> 10 c.c. at 0.0001 *M*, and water 25 c.c. at 14° C. Sections 0.24 to 0.26 mm. in thickness.

TABLE VII.

	0.01 <i>M</i> .		0.001 <i>M</i> .		0.0001 <i>M</i> .	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
KCl .....	1,300	1,690	1,000	1,300	970	1,200
CaCl <sub>2</sub> .....	750	940	860	1,000	970	1,220
HCl .....	1,760	5,750	1,580	2,025	700	815
Salt solution .....					650	725
Water .....					1,120	

It is to be recalled that this gelatine had a Ph value of 5.2 and that the incorporated salts a Ph of about 5.8 to 6, so that when the dried sections were placed in a salt solution of this constitution the swelling was less than in water and that a similar decrease was noted in the calcium and potassium solutions in 0.0001 *M* and 0.001 *M* solutions and in the calcium solution at 0.01 *M* also. Increases

took place in the potassium solution at PH of 6.6 and in the acid at PH of 3 and 2. It is evident, without a detailed analysis of these results, that many features beside hydrogen ion concentration are involved.

All of the foregoing tests had as their chief purpose the determination of the reactions of plasmatic constituents and the experiments were extended to include the swellings of a biocolloid including both pentosans and gelatine with the addition of salts, but without the third colloidal component, the soaps, which we now have ample reason to believe play a very important part in the mechanism of living matter. A mixture of gelatine 3 parts and agar 2 parts was made in the usual manner with the addition of salts as in the preparations of agar and of gelatine separately. The increases when hydrated in various solutions were as noted in Table VIII.

TABLE VIII.

	0.01 <i>M.</i>		0.001 <i>M.</i>		0.0001 <i>M.</i>	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
KCl .....	900	1,030	1,500	1,780	1,240	1,500
NaCl .....	1,240	1,450	1,240	1,450	1,600	1,970
CaCl <sub>2</sub> .....	800	855	820	940	1,060	1,190
HCl .....	1,735	2,070	700	745	1,140	1,260
Salt solution .....					900	1,030
Water .....					1,250	1,500

The more prominent reactions are those of the gelatine element as would be expected in accordance with which the highest concentration is in the most concentrated solution of the acid. It is notable that as in the gelatine-agar salt-free plate depression occurred in acid at PH 3 and that at PH 4.2 the swelling was still below that of water. Practically applied to the living cell this would mean that hydration lessened with increasing acidity to the region of PH 3 beyond which the active cell would rarely go. Sodium and calcium exerted similar effects, but the swelling in potassium at 0.0001 *M* was equivalent to that in water while at 0.001 *M* it was greater. A review of the results presented in this paper would show many special results from the action of this salt.

The recently published results of experiments upon the coagu-

lating action of neutral salts upon plasmatic colloids by Tadokoro furnish some important collateral conclusions. Tadokoro found that all chlorides of aluminium, barium, strontium, calcium and magnesium cause coagulation of plasmatic colloids in an increasing series in the order given in concentrations from  $N/200$  to  $N/10$ , and that KCl exerted a stronger action than NaCl. Many profitable comparisons may be made between the results of his tests of the action of salts upon crushed galls and of the swelling reactions of biocolloids given in the present paper.<sup>8</sup> Furthermore both afford many parallels with the swelling reactions which were obtained by the extension of my experiments to include the measurement of the action of the salts, particularly the chlorides, upon living and dead cell-masses.

The first material tested was taken from the large "prop" roots of corn plants a meter in height growing in the garden at Carmel. Root-hairs were only sparingly developed on a terminal section 3 to 5 cm. long, and sections 4 or 5 mm. in length including the tip were taken and freed as completely as possible from particles adherent from the loose sandy soil in which they grew. The average thickness of trios placed under the auxograph ranged from 2 to 2.5 cm. Such sections dried down to thickness of 0.3 to 0.4 mm. when placed between sheets of blotting paper.

Such sections of living material made practically all of the changes in volume indicated within eight to fifteen minutes, the speediest action taking place in the acid and the slowest recorded being in the potassium solution, although this matter is partly a function of the size or length of the sections. In illustration of the swelling and shrinkage it may be cited that in the acid the sections return to normal size in about 2 hours. Shrinkage to original thickness may in some cases take place within a day, although twice this period elapses in other instances.

The results of the auxographic measurements are given as average percentages of original thickness in Table IX.

If the facts in this table are taken for comparison with those

<sup>8</sup> Tadokoro, T., "Kolloidchemische Forschungen ueber das Pflanzenplasma," *Jour. Coll. of Agric.*, Hokkaido Imp. Univ., Sapporo, Japan, 7: part 5, 144-182, 1919.

obtained from the salted biocolloid, disregarding possible osmotic effects, it will be seen that hydration in both potassium and sodium is greater than in water, magnesium equivalent, the swelling less in calcium and acid, indicating that the cell-colloids are dominantly

TABLE IX.

HYDRATION OF ROOTS OF *Zea mays*, LIVING AND DRIED, AT 15° C.

	Living.		Dried.	
	0.01 <i>M</i> Per Cent.	0.0002 <i>M</i> Per Cent.	0.01 <i>M</i> Per Cent.	0.0002 <i>M</i> Per Cent.
NaCl .....	4.5	4, 4, 4	94	120
MgCl <sub>2</sub> .....	3.5		112	
CaCl <sub>2</sub> .....	0			
Balanced solution .....	1		100	
HCl .....	2.1		88	
KCl .....	4			
Water .....	3.4		65	

pentosans. The dried and dead material showed increased hydration capacity over water in all solutions except that of calcium, measurements which may be taken to be free from the major part of the errors introduced by the osmotic and plasmolytic effects in the living material. It is notable also that when calcium was balanced with sodium the swelling was at a minimum in the living material but it rose above that in water in the dried cell-masses, but did not attain the maximum value for the series which was shown by the calcium solution at 0.0002 *M*. The dried material presents a series of hydration reactions which suggest those of an agar-gelatine mixture.

The smaller actively growing roots of the same maize plants were subjected to comparative tests in a living condition only. Swelling was most in acid and least in water, the series being water = NaCl, balanced solution, CaCl<sub>2</sub> = KCl, HCl, as contrasted with the larger roots in which the series was CaCl<sub>2</sub>, balanced solution, HCl, KCl, and NaCl as given in Table X.

Seeds furnished by Dr. Fawcett, of the Citrus Experiment Station at Riverside, California, were sprouted in a chamber at 23° to 25° C., by being placed in a moist sand bed. When the roots had attained a length of 1 to 3 cm. the apical portions 3 or 4 mm. in

TABLE X.

HYDRATION OF SMALL MAIZE ROOTS IN VARIOUS SOLUTIONS AT 15° C.

	0.01 M.
NaCl .....	10
Balanced solution .....	18
CaCl <sub>2</sub> .....	20
HCl .....	30
KCl .....	20
Water .....	10

length were cut off, placed in trios in glass dishes, covered with a triangular sheet of glass from which bearings were taken by auxographs in a room at 15° C. The swellings in percentages of original thickness were as in Table XI.

TABLE XI.

	0.01 M.	0.0002 M.	0.0001 M.
NaCl .....	0.7		0.8
Balanced solution .....	1		2.2
CaCl <sub>2</sub> .....		0.9	
KCl .....	1		1
HCl .....	0.5		0.7
Sea water .....	1.5		2
Water .....			2

The series at 0.01 M was HCl, NaCl, KCl=balanced solution, sea-water, water. In the more attenuated solutions the series was HCl, NaCl, CaCl<sub>2</sub>, KCl, sea-water, water, balanced solution, only the last named producing a hydration greater than in water. The series is suggestive of the action of a dominantly pentosan bio-colloid.

Some plants of *Fragaria* (strawberry) which were growing in an injuriously saline soil were transferred to a sand bed and roots grown in both sub-strata were available for testing. The differences which might be induced by these divergent factors are well illustrated by the measurements given in Table XII.

TABLE XII.

	Grown in Sand.	Grown in Soil.
NaCl 0.01M .....	50	10
Balanced solution 0.01M .....	45	5
HCl 0.01N .....	15	8
Sea-water 0.01M .....	20	5
Water .....	30	10

The hydration capacity of the roots grown in saline soil was less throughout than that of the roots grown in sand in which the salts introduced by the water of irrigation were much attenuated. Other differences suggest that the roots in the saline soil were more highly proteinaceous and also had a higher incorporated salt content.

Still another test was made with sections taken from the joints of *Opuntia* growing near the Desert Laboratory in February, 1921. The course of change in the chemical composition of this material is known in some detail. The pentosan constituent of the cell-colloids reaches a maximum earlier in the winter and was lessening at the time of the experiments but would still be so large as to make for a dominantly pentosan colloid. Swellings as in Table XIII were exhibited by two samples which are given in separate columns.

TABLE XIII.

Water .....	164	168
KOH 0.01 <i>N</i> .....	177	175
KOH 0.001 <i>N</i> .....	152	158
KOH 0.0001 <i>N</i> .....	169	153
HCl 0.01 <i>N</i> .....	146	136
HCl 0.001 <i>N</i> .....	172	161
HCl 0.0001 <i>N</i> .....	150	150
KCl 0.01 <i>M</i> .....	150	153
KCl 0.001 <i>M</i> .....		146
KCl 0.0001 <i>M</i> .....		179

Swellings greater than in water are induced by KOH with a PH value of 12, in KCl with a PH value of 5.7 and by HCl at 0.001 *N* with a PH value of 5.7.

Here as in all tests of living material the measurements are complicated by osmotic effects, although it is to be noted that the final swellings in *Opuntia* are reached after immersion for a day and the results are more clearly imbibitional than in any of the other material used.

#### GENERAL CONSIDERATIONS.

The following generalizations may be made upon the basis of the experimental results described in the foregoing paper.

1. The strong metallic bases which were found to lessen the swelling of agar to a degree corresponding to their relative posi-

tions in the electromotive series when used as hydroxides, give the same relative action when used as chlorides. The series runs Ca, K, Na, the greatest retardation being effected by the calcium and the least by sodium, when used at concentrations of 0.01 *M*.

2. Reversed effects by which hydration of agar is increased are shown by the hydroxides at 0.001 *N*, as described in a previous contribution, but no well-defined differences among the bases used could be made out. Similar reversed effects were exhibited by the chlorides of calcium, magnesium, potassium and sodium at 0.0001 *M* and by potassium and sodium in concentrations as great as 0.001 *M*.

3. Purified agar used in the experiments has a PH value of 6.5, also swells more in HCl at a PH value of 4.2 than in water, a statement to be applied in correction of various conclusions in previous papers.

4. Purified agar shows exaggerated swellings in a series of acid, salt and hydroxide solutions in which the hydrogen ion concentration ranges from PH 4.2 to 11.

5. Purified agar also shows exaggerated swellings in sodium and potassium nitrates at 0.0001 *M* but not in the sulphates.

6. Of the chlorides of calcium and potassium and hydrochloric acid at concentrations from 0.01 *M* to 0.0001 *M* only KCl at 0.001 and 0.0001 *M* increase the swelling of an agar-gelatine mixture. In a similar series only KCl at 0.0001 *M* increases swelling in a gelatine-agar mixture.

7. Agar plates with included chlorides at concentrations increasing swelling, when applied as hydrating solutions showed exaggerated swelling in HCl, NaCl, KCl at 0.0001 *M*, but a lessened swelling in CaCl<sub>2</sub> and MgCl<sub>2</sub> at this concentration.

8. Gelatine plates with incorporated salts as above showed swelling in HCl increasing with the concentration beginning with the 0.001 *M* solution, in reverse of the action of the CaCl<sub>2</sub> solution which was greatest but still less than in water until at 0.0001 *M*. Swelling in KCl did not exceed that in water until a concentration of 0.01 *M* was reached.

9. The maximum swelling of a gelatine (3 parts)-agar (2 parts) plate is greatest in HCl 0.01 *N*, KCl 0.001 *M* and CaCl<sub>2</sub> at 0.0001 *M*.



10. Different ecological types of roots of maize show different hydration reactions to the solutions used in hydration tests of colloids as noted in the foregoing paragraphs.

11. Roots of strawberry show differing hydration reactions when grown in saline soils and in sand.

12. Roots of orange seedlings show lessened hydration in acid solutions and their hydration was lessened in all solutions except balanced solutions of sea-water and of sodium and calcium chloride.

13. Swellings of sections of joints of *Opuntia* were greatest in KOH at 0.01 *N*, HCl at 0.001 *N* and KCl at 0.0001 *M*, all producing effects in excess of the swelling in water.

14. The changes in volume of living cell-masses in hydrating solutions include osmotic-plasmolytic effects in the alterations of the volume of the included cells. The hydration of dead cell-masses includes possible osmotic action of cell-walls.

15. The hydration reactions described in this paper may include coagulation effects when the higher concentrations were applied to the biocolloids, similar to those of the plasmatic colloids. Actual effects of balanced solutions are clearly defined in the hydration of agar, and some suggestions of similar action in the biocolloids arise from the measurements of swelling of the biocolloids described.